**Table 3**
Significantly dysregulated pathways in OA

| Rank | Name | pSize | NDK | pNDE | tA | pPERT | pG | pGFdr | pGFWER | Status |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | PI3K Akt signaling pathway | 337 | 21 | 2.96E-08 | -5.30E+01 | 1.20E-02 | 8.09E-09 | 7.93E-07 | 7.93E-07 | Inhibited |
| 2 | Circadian rhythm | 30 | 7 | 2.04E-07 | -7.24E+00 | 5.00E-02 | 1.98E-07 | 9.70E-06 | 1.94E-05 | Inhibited |
| 3 | HIF-1-alpha transcription factor network | 63 | 10 | 2.50E-08 | 1.55E+00 | 6.68E-01 | 3.16E-07 | 3.59E-05 | 4.49E-05 | Activated |
| 4 | Validated transcriptional targets of AP1 family members Fra1 and Fra2 | 37 | 7 | 9.46E-07 | 6.56E+00 | 2.90E-02 | 5.05E-07 | 3.59E-05 | 7.17E-05 | Activated |
| 5 | HIF-1 signaling pathway | 108 | 11 | 5.39E-07 | 1.13E+01 | 3.86E-01 | 3.41E-06 | 1.11E-04 | 5.34E-04 | Activated |
| 6 | C-MYB transcription factor network | 77 | 10 | 1.79E-07 | 1.03E+00 | 8.88E-01 | 2.65E-06 | 1.26E-04 | 3.77E-04 | Activated |
| 7 | Acute myeloid leukemia | 56 | 7 | 1.68E-05 | 2.28E+00 | 6.82E-01 | 1.42E-04 | 2.79E-03 | 1.39E-02 | Activated |
| 8 | ECM-receptor interaction | 86 | 6 | 1.64E-03 | -1.34E+01 | 7.00E-01 | 1.42F-04 | 2.79E-03 | 1.40E-02 | Inhibited |
| 9 | IL6-mediated signaling events | 47 | 6 | 6.04E-05 | 7.06E+00 | 3.84E-01 | 2.71E-04 | 9.61E-03 | 3.85E-02 | Activated |
| 10 | HTLV-I infection | 260 | 13 | 1.28E-04 | -8.73E+00 | 2.93E-01 | 4.20E-04 | 1.19E-02 | 4.74E-02 | Inhibited |

*pSize*: number of genes in the pathway; *NDE*: number of DE genes in pathway; *pNDE*: Probability to observe at least NDE genes in the pathway; *tA*: observed total perturbation accumulation in the pathway; *pPERT*: probability to observe a total accumulation more extreme than tA by chance; *pG*: p-value obtained by combinging pNDE and pPERT; *pGFdr*: False discovery rate; *pGFWER:* Bonferroni adjusted global p-values; Status: Direction in which the pathway is perturbed.

## 412
## POWERFUL DETECTION OF OSTEOARTHRITIS SUSCEPTIBILITY LOCI BY COMPREHENSIVE EXAMINATION OF CLINICALLY IMPORTANT ENDOPHENOTYPES

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**Purpose:** Osteoarthritis (OA) is a highly heterogeneous disease characterised by variable clinical features with possibly different genetic aetiologies. Thus far, the few genetic variants that have been robustly associated with broad definitions of OA (n = 13 in Europeans) explain only a small proportion of its heritability. Our aim is to identify novel OA susceptibility variants by examining an expanded set of more homogeneous, radiographically-derived OA endophenotypes relating to joint morphology, specific anatomic pattern of joint involvement, severity and bone response in OA.
**Methods:** 2,000 knee and 2,000 hip OA cases with radiographs have been genotyped as part of the arcOGEN study on the Illumina Human 610-Quad Beadchip and Illumina HumanOmniExpress Beadchip arrays. Variables relating to joint morphology, specific anatomic pattern of joint involvement, severity and bone responses in OA were extracted from digitised radiographs. Following 1000 Genomes Project-based imputation and stringent quality control >7 million variants were tested for association with each phenotype. Logistic regression was used for binary variables and linear regression was used for continuous variables adjusted for gender. Fixed-effects meta-analysis was used to combine the results from the two genome-wide association studies (GWAS).
**Results:** Our results indicate that the study of endophenotypes in OA has the potential to dramatically enhance power to detect OA-relevant associations. For example analysis by knee compartment involvement vs population-based controls yielded 25 independent loci for knee OA at $p < 1 \times 10^{-6}$ in contrast to 1 locus detected for knee OA vs controls in the equivalent binary trait GWAS. In hip OA endophenotype analyses several promising signals were identified some of which are found near genes that are very plausible biological candidates for OA. For example in the analysis of atrophic vs hypertrophic hip OA response a strong signal (OR [95% CI] = 2.03 [1.57–2.63], $p = 2.5 \times 10^{-8}$) was detected in the G protein-coupled receptor, GPR98. Polymorphisms in GPR98 and another G protein-coupled receptor (GPR48) have been associated with osteoporotic fracture and low bone mineral density respectively and gpr98 knockout mice have a low bone mass phenotype. Pattern of hip migration (axial/medial vs non-axial/medial migration) shows strong association with variants in LRCH1 (rs754106, p=2.9x10-7) previously suggestively associated with OA and BMP1 (chr8: 22065846, $p = 2.6 \times 10^{-7}$) which induces bone and cartilage development. From the hip morphology studies the strongest signals were detected in the analysis of femoral neck-length-to-width ratio (rs3112954, located in an intron of ZNF385B, $p = 6.5 \times 10^{-8}$ and rs11695150, $p = 8.8 \times 10^{-8}$ located in an intron of PP1R21).
**Conclusions:** Through a comprehensive examination of radiographically-derived, OA-related phenotypes we have identified several promising signals that point to novel and biologically plausible genes for OA. Our results indicate that, in a heterogeneous disease like OA the study of narrower phenotype definitions closer to the biology of the disease has the potential to dramatically enhance power to detect OA-relevant associations and yield an unprecedented amount of information on OA susceptibility genes. Further replication is required to boost power and validate these associations.

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## NEW FUNCTIONAL MICROSATELLITE ASSOCIATED WITH OSTEOARTHRITIS SUSCEPTIBILITY

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**Purpose:** Considering that two functional microsatellites are associated with OA, in the *BMP5* and *ASPN* genes, and that their effects are poorly represented by neighboring SNPs, we hypothesized that other genetic variants with these characteristics could contribute to OA susceptibility and have escaped detection in GWAS. To test this hypothesis we searched the bibliography identifying six additional functional microsatellites. Two had been already associated with OA in studies with less than 150 patients, in the interleukin (*IL10*) and calcitonin (*CA*) genes, whereas a second microsatellite in *IL10* was studied, but did not show association. Three other functional microsatellites, in the estrogen receptor 2 (*ESR2*), the tyroxine hydroxylase (*TH*) and the macrophage migration inhibitory factor (*MIF*) genes, have never been studied in OA. We analyzed these six microsatellites in a large case-control study.
**Methods:** The six functional microsatellites were amplified with labeled primers in samples from 3557 patients with OA (1775 of knee OA and 1782 of hip OA) and in 1878 healthy controls of similar age. All patients and controls were of European Caucasian ancestry either from the UK, Greece or Spain. A subset of samples was genotyped twice for quality control. POWERMARKER, CLUMP and GENEPOP were used for analysis. Genotypes of SNPs in the neighborhood of the *BMP5*, *ASPN*, *MIF* and *TH* microsatellites were obtained from the arcOGEN GWAS for the UK samples included in this study. They were used to assess imputation of microsatellite genotypes with the Beagle and IMPUTE algorithms.
**Results:** Genotypes of the *CA* microsatellite were inconsistent in replication and discarded. Reproducible genotypes were obtained for the other five microsatellites with call rates >0.93. The *MIF* microsatellite showed five alleles (from 4 to 8 repeats) with homogeneous frequency distribution in the three populations. This microsatellite was associated with hip OA with contrasting effects in women (*P* of the Mantel-Haenszel analysis = 0.018) and men ($P_{M\text{-}H} = 0.029$): the 6 repeat allele

was in excess in control women relative to women with hip OA, whereas it was in excess in men with hip OA relative to control men (Table 1). These differences showed a consistent direction in the three studied populations although they were only significant in the Spanish subjects ($P = 0.0029$ in women and $P = 0.021$ in men). In addition, the *TH* microsatellite showed 8 alleles (from 4 to 11 repeats) with heterogeneous frequency distribution in the three populations and association with knee OA in the Spanish samples ($P = 0.008$) that was not replicated in the other two populations and that resulted in a non-significant association in the global analysis of the three populations ($P_{M\text{-}H} = 0.12$). The other three microsatellites did not show association in any of the analyses. To assess the possibility of imputing microsatellite genotypes from neighboring SNP, we applied a protocol of training with 90% of up to 1100 samples for which microsatellite and GWAS data were available to impute the 10% remaining and repeated this process 10 times. The best results were obtained with different algorithms for different microsatellites, but correct genotypes were less than 90 % and most often less than 80 %.

**Conclusions:** A new functional microsatellite in *MIF* has been found associated with hip OA, showing an opposed direction of association in women and men. This microsatellite is in a gene, *MIF*, of potential relevance for OA as a cytokine produced by chondrocytes in repair and inflammatory responses. This microsatellite together with those in *BMP5*, *ASPN*, and perhaps in *TH*, require further study to definitively establish their status. Unfortunately, imputation of their genotypes from GWAS data was too inaccurate in our tests.

**Allele frequencies of the MIF microsatellite showing association with hip OA**

| | women | | | | | | men | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Spain | | UK | | Greece | | Spain | | UK | | Greece | |
| Allele | CRL | HipOA | CRL | HipOA | CRL | HipOA | CRL | HipOA | CRL | HipOA | CRL | HipOA |
| 5 | 178 (25.7) | 217 (29.7) | 169 (24.0) | 284 (23.8) | 111 (26.1) | 35 (26.1) | 270 (29.5) | 162 (25.9) | 178 (26.4) | 207 (26.0) | 77 (35.0) | 10 (21.7) |
| 6 | 455 (65.8) | 424 (58.1) | 453 (64.3) | 761 (63.7) | 282 (66.2) | 81 (60.4) | 544 (59.5) | 409 (65.3) | 409 (60.7) | 501 (62.9) | 123 (55.9) | 32 (69.6) |
| 7 | 59 (8.5) | 88 (12.1) | 81 (11.5) | 148 (12.4) | 33 (7.7) | 18 (13.4) | 100 (10.9) | 55 (8.8) | 85 (12.6) | 86 (10.8) | 20 (9.1) | 4 (8.7) |
| 8 | 0 | 1 (0.1) | 1 (0.1) | 1 (0.1) | 0 | 0 | 0 | 0 | 2 (0.3) | 2 (0.3) | 0 | 0 |
| Total | 692 | 730 | 704 | 1194 | 426 | 134 | 914 | 626 | 674 | 796 | 220 | 46 |

## 414
## CHARACTERISATION OF THE CARTILAGE DNA METHYLOME IN KNEE AND HIP OSTEOARTHRITIS USING HIGH-DENSITY GENOME-WIDE ANALYSIS

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**Purpose:** Deregulation of DNA methylation is known to play a fundamental role in the onset and progression of common diseases. In osteoarthritis (OA) it is proposed that altered gene expression mediated through aberrant DNA methylation is involved in the shift in the balance of cartilage maintenance towards degradation. For example, candidate gene studies have shown that over-expression of metalloproteinases in OA chondrocytes can occur as a result of altered DNA methylation in the promoters of the genes coding for these enzymes. The aim of this study was to characterise the DNA methylome in hip and knee OA at the genome-wide level. This would provide the first comparison between OA hip chondrocytes and healthy hip chondrocytes, as well as the first comparison between OA hip and OA knee chondrocytes.

**Methods:** Cartilage was obtained from the hip ($n = 23$) or knee ($n = 77$) joints of patients who had undergone joint replacement surgery as a result of primary OA. Cartilage obtained from patients who had undergone hip replacement due to a neck of femur (NOF) fracture were used as non-OA controls ($n = 21$). DNA extracted from the cartilage was then bisulphite converted before being taken forward for methylation analysis. This was performed using the Illumina 450k BeadArray, which measures the level of DNA methylation at approximately 480,000 CpG sites throughout the human genome. To identify differentially methylated loci (DMLs) the average methylation value was compared between the groups of interest (for example, OA hip versus NOF). A locus was deemed significant if there was at least a 10% difference in methylation between the two groups, and a Benjamini-Hochberg corrected P value of $<0.05$.

**Results:** We identified that OA hip and healthy hip controls have a unique methylation profile, with a total of 3607 DMLs identified between the two groups. There was an enrichment of genes coding for proteins involved in the catabolic/anabolic balance of cartilage tissue homeostasis, including enzymes that degrade cartilage and members of the TGFβ superfamily. A total of 6019 DMLs were identified between OA knee and OA hip samples. These included genes involved in skeletal development and, intriguingly, genes that reside in regions of the genome that are genetically associated with increased risk of OA development. Interestingly we observed that the OA hip samples clustered into two groups based on their DNA methylation profile and that the clusters were distinguishable due to an enrichment of genes within pathways involved in inflammation and immunity. Likewise, we observed that OA knee samples also cluster into two groups.

**Conclusions:** Our study, which is the most powerful cartilage methylome analysis yet performed in OA, demonstrates that the molecular characterisation of DNA methylation differences is a powerful tool for identifying pathways involved in the initiation and/or progression of the disease. There are striking differences in the methylation profile between OA and OA-free cartilage, and between OA hip and OA knee cartilage. This latter observation reinforces the contrasting nature of OA progression between these two joints. Finally, the identification of subclusters of patients within the OA hip and within the OA knee groups emphasizes the complexity of the pathogenesis of this common arthritis.

## 415
## GLOBAL GENE EXPRESSION CHANGES FOLLOWING TRAUMATIC MECHANICAL IMPACT ON BOVINE ARTICULAR CARTILAGE

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**Purpose:** Osteoarthritis (OA) is a debilitating and degenerative joint disease that affects >15% of the population in the U.S. While aging is highly correlated to the incidence and severity of OA, posttraumatic OA (PT-OA) accounts for a significant fraction, ~12%, of all cases of OA. There are several *in vitro* models of PT-OA that deliver single, high energy impacts to articular cartilage. We have recently described the characteristics of a spring-loaded impactor that produces early degenerative changes in adult bovine articular cartilage after both high, fissuring impacts (36 MPa) and low or non-fissuring impacts (17 MPa). In this study we aimed to analyze the genes differentially expressed within the first week after applying mechanical trauma to articular cartilage. Investigating the global gene expression profiles will allow us to identify functional gene groups affected by mechanical injury that could be overlooked by candidate gene analysis. These gene expression changes are likely to provide insights into the early etiological mechanisms of PT-OA that may lead to identification of early therapeutic targets.

**Methods:** Adult bovine articular cartilage plugs harvested from the patellofemoral groove were either unimpacted (CTRL) or impacted with loads of 17 or 36 MPa. Cartilage explants were cultured in media and then collected at several time points (1, 3 and 7 days). Total RNA was isolated from 3 independent experiments. Global gene expression changes were determined by microarray analysis using bovine Gene Chip v1 Affymetrix arrays and the J5 algorithm within the web application caGEDA developed at University of Pittsburgh Genomics and Proteomics Core. The resulting gene lists, ranked by their J5 score, were then uploaded into the Ingenuity Pathway Analysis (IPA) for identification of relevant biological groups.